

Phenotypic and genotypic analysis of bio-serotypes of *Yersinia enterocolitica* from various sources in Brazil

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Abstract

Introduction: *Yersinia enterocolitica* is a well-known foodborne pathogen widely distributed in nature with high public health relevance, especially in Europe.

Methodology: This study aimed to analyze the pathogenic potential of *Y. enterocolitica* isolated strains from human, animal, food, and environmental sources and from different regions of Brazil by detecting virulence genes *inv*, *ail*, *ystA*, and *virF* through polymerase chain reaction (PCR), phenotypic tests, and antimicrobial susceptibility analysis. Pulsed-field gel electrophoresis (PFGE) was used for the assessment of phylogenetic diversity.

Results: All virulence genes were detected in 11/60 (18%) strains of serotype O:3, biotype 4 isolated from human and animal sources. Ten human strains (4/O:3) presented three chromosomal virulence genes, and nine strains of biotype 1A presented the *inv* gene. Six (10%) strains were resistant to sulfamethoxazole-trimethoprim, seven (12%) to tetracycline, and one (2%) to amikacin, all of which are used to treat yersiniosis. AMP-CEF-SXT was the predominant resistance profile. PFGE analysis revealed 36 unique pulsotypes, grouped into nine clusters (A to I) with similarity $\geq 85\%$, generating a diversity discriminatory index of 0.957. Cluster A comprised all bio-serotype 4/O:3 strains isolated from animal and humans sources.

Conclusions: This study shows the existence of strains with the same genotypic profiles, bearing all virulence genes, from human and animal sources, circulating among several Brazilian states. This supports the hypothesis that swine is likely to serve as a main element in *Y. enterocolitica* transmission to humans in Brazil, and it could become a potential threat to public health as in Europe.

Key words: *Yersinia*; zoonosis; antibiotics; PCR; PFGE.

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Introduction

Yersinia enterocolitica was of clinical and epidemiological interest in the 1970s, evidenced by the significant increase of studies published around the world [1]. It is widely distributed in nature in aquatic and animal reservoirs, with swine serving as a major reservoir for human pathogenic strains [2]. Yersiniosis is a zoonotic bacterial disease with high public health relevance, especially in Europe due to its high levels of occurrence, where it is the third most common bacterial enteric disease [3-4].

In Brazil, several studies on *Y. enterocolitica* and other *Yersinia* species show its isolation from human, animal, food, and environmental sources; occurrences

are most frequently reported in sporadic cases [5]. Brazil does not have official data about the incidence of this pathogen. The sporadic cases have been reported in only a few studies that have been published in scientific journals around the world.

Regarding clinical aspects, *Y. enterocolitica* O:3 may cause a variety of gastrointestinal problems, such as acute diarrhea, terminal ileitis, and mesenteric lymphadenitis. The pathogenicity and virulence mechanisms are still complex, and various chromosomal and extra-chromosomal factors have already been described [2]. The *ail*, *inv*, and *yst* genes, located in the chromosome of pathogenic *Y. enterocolitica* strains, are the most frequently used

chromosomal targets [6]. The *inv* (invasive gene) and *ail* (attachment invasion locus) genes are responsible for the production of an invasin and an outer membrane protein called Ail, respectively, which enable the bacteria translocation through the intestinal epithelium, cell fixation, and subsequent invasion. The role of the *ystA* gene (yersinia stable toxin) in the production of thermostable enterotoxin, causing diarrhea, has also been emphasized [7].

Another virulence factor in *Y. enterocolitica* O:3 is a plasmid called pYV (plasmid *Yersinia* virulence) [8]. It allows the microorganism to survive and multiply in lymphatic tissues and encodes the production of several proteins called Yops (*Yersinia* outer proteins). They play a major role in yersinial virulence, whose effects on macrophages and polymorphonuclear leukocytes include the inhibition of phagocytosis and respiratory burst [2]. In plasmids, the locus *virF* contains information on Yops transcription [9].

In the last decades, several different DNA-based methods for epidemiological typing have been used in epidemiological outbreak studies of *Y. enterocolitica* [5]. PFGE is the gold standard for bacteria and is used both for studies of hospital outbreaks and for the comparison of bacterial populations involving microorganisms of different countries or regions [10].

The aim of this study was to analyze the virulence genes, antimicrobial susceptibility behavior, and genotypes of *Y. enterocolitica* Brazilian strains isolated from humans and animals, which could act as a reservoir and a transmission route.

Methodology

Bacterial strains and bacteriological analysis

A total of 60 *Y. enterocolitica* strains were randomly selected. The isolate sources included 31 strains from tonsillar and tongue surfaces of healthy swine; 10 from slaughterhouses in Campinas city isolated between 2007 and 2008; 5 strains from food isolated in São Paulo state (SP) (2008–2011); and 14 isolates from humans with various clinical circumstances coming from Bahia (BA), Minas Gerais (MG), Paraná (PR), Rio de Janeiro (RJ), and Santa Catarina states (SC) between 2005 and 2011.

The strains belonged to the *Listeria* collection (CLIST), Laboratory of Bacterial Zoonosis at Oswaldo Cruz Institute, Fiocruz, Rio de Janeiro.

Y. enterocolitica biotyping was performed according to Wauters (1981) [11] and Mollaret *et al.* (1990) [12]. Serotyping was performed by slide agglutination and typed according to Wimblad's

(1968) [13] O antisera system prepared in rabbits in the laboratory [14].

Phenotypic tests for detection of virulent plasmid

To assess the presence of the virulent plasmid, the following phenotypic tests were performed: autoagglutination phenotypic tests at 37°C [15], binding of crystal violet [16], absorption of Congo red in the Congo red-magnesium oxalate agar medium (CRMOX) [17], and the activity of pyrazinamidase [18]. All phenotypic tests, except for the pyrazinamidase activity test, were performed at 28°C and 37°C.

Antimicrobial susceptibility

Antimicrobial susceptibility tests were carried out using the disk diffusion method as recommended by the Clinical and Laboratory Standards Institute, 2011 [19]. Twelve antimicrobials (Oxoid, Basingstoke, UK) were tested: ampicillin (AMP) 10 µg, cephalotin 30 µg (CEF), cefoxitin (FOX) 30 µg, amikacin (AMK) 30 mg, sulfamethoxazole (SFT) 25 µg, gentamicin (GEN) 10 µg, tetracycline (TET) 30 µg, chloramphenicol (CLO) 30 µg, imipenem (IPM) 10 µg, sulfamethoxazole-trimethoprim (SXT) 25 µg, ciprofloxacin (CIP) 5 µg, and trimethoprim (TMP) 5 µg. The reference strains *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were used as the quality control of all antimicrobials. *Pseudomonas aeruginosa* ATCC 27853 was used as the quality control for amikacin and ciprofloxacin.

Detection of virulence genes

The presence of *inv*, *ail*, *virF*, and *ystA* genes was analyzed through polymerase chain reaction (PCR) with their respective primers (Table 1) using Platinum PCR Super Mix Kit (Invitrogen, Carlsbad, USA). The conditions for performing PCR reactions were followed according to the reference of each gene in Table 1. DNA extraction was performed using a DNA extraction kit (DNeasy Blood & Tissue Kit; Qiagen, Hilden, Germany), following the manufacturer's guidelines.

Pulsed field gel electrophoresis (PFGE)

PFGE was performed according to the methodology recommended by Pulsenet [23] for *Yersinia pestis*. The cell suspension of the strains was made with cell suspension buffer (CSB – 100 mM Tris; 100 mM EDTA; pH 8.0). The agarose plugs were made with 400 µL of 1% SeaKem Gold (Cambrex, Rockland, USA) and 1% SDS agarose.

Table 1. Primers of virulence genes *inv*, *ail*, *ystA* and *virF* and their respective amplicon

Gene	Name	Sequence (5'–3')	Amplicon (bp)	Reference
<i>inv</i>	YC1 (F)	CTG TGG GGA GAG TGG GGA AGT TTG G	570	Rasmussen et al. (1994) [20]
	YC2 (R)	GAA CTG CTT GAA TCC CTG AAA ACC G		
<i>ail</i>	Ail1 (F)	ACT CGA TGA TAA CTG GGG AG	170	Nakajima et al. (1992) [21]
	Ail2 (R)	CCC CCA GTA ATC CAT AAA GG		
<i>ystA</i>	Pr2a (F)	A ATG CTG TCT TCA TTT GGA GCA	145	Ibrahim et al. (1997) [22]
	Pr2c (R)	ATC CCA ATC ACT ACT GAC TTC		
<i>virF</i>	VirF1 (F)	TCA TGG CAG AAC AGC AGT CAG	590	Wren & Tabaqchali (1990) [9]
	VirF2 (F)	ACT CAT CTT ACC ATT AAG AAG		

Table 2. Results of antimicrobial resistance (AR), PCR of virulence genes and phenotypic tests of 60 *Y. enterocolitica* strains analyzed

Strain	Source	Date	AR	Virulence genes	Phenotypic tests	Serotype / Biotype
YE1	Animal	2007	AMP, CEF, SFT, TET, SXT	-	CV	O:3/4
YE2	Animal	2007	AMP, CEF	-	CV	O:3/4
YE3	Animal	2007	AMP, CEF, SFT, TET	-	-	O:3/4
YE4	Animal	2007	AMP, CEF	<i>inv, ail, ystA, virF</i>	Autoag, CV, CRMOX	O:3/4
YE5	Animal	2007	AMP, CEF, SXT, TMP	-	-	O:3/4
YE6	Animal	2007	AMP, CEF, SFT	-	-	O:3/4
YE7	Animal	2007	AMP, CEF, SFT	-	-	O:3/4
YE8	Animal	2007	AMP, CEF, SFT	-	-	O:3/4
YE9	Animal	2007	AMP, CEF, SFT, TET	-	CRMOX	O:3/4
YE10	Animal	2007	AMP, CEF, SFT,	-	-	O:3/4
YE11	Animal	2007	AMP, CEF, SFT,	-	-	O:3/4
YE12	Animal	2007	AMP, CEF, AMK, SFT,	-	-	O:3/4
YE13	Animal	2007	AMP, CEF	<i>inv, ail, ystA, virF</i>	Autoag, CV, CRMOX	O:3/4
YE14	Animal	2007	AMP, CEF, SFT,	<i>inv, ail, ystA, virF</i>	Autoag, CV, CRMOX	O:3/4
YE15	Animal	2007	AMP, CEF	<i>inv, ail, ystA, virF</i>	Autoag, CV, CRMOX	O:3/4
YE16	Animal	2008	AMP, CEF	-	CRMOX	O:3/4
YE17	Animal	2008	AMP, CEF, TET, SXT	-	-	O:3/4
YE18	Animal	2008	AMP, CEF	-	CRMOX	O:3/4
YE19	Animal	2008	AMP, CEF, SFT	-	CRMOX	O:3/4
YE20	Animal	2008	AMP, CEF, SFT, TET	-	CRMOX	O:3/4
YE21	Animal	2008	AMP, CEF, SFT	<i>inv, ail, ystA, virF</i>	Autoag, CV, CRMOX	O:3/4
YE22	Animal	2008	AMP	<i>inv, ail, ystA, virF</i>	Autoag, CV, CRMOX	O:3/4
YE23	Animal	2008	AMP, CEF, SFT	<i>inv, ail, ystA, virF</i>	Autoag, CV, CRMOX	O:3/4
YE24	Animal	2008	AMP, CEF	<i>inv, ail, ystA, virF</i>	Autoag, CV, CRMOX	O:3/4
YE25	Animal	2008	AMP, CEF, SFT	-	-	O:3/4
YE26	Animal	2008	AMP, SFT	<i>inv, ail, ystA, virF</i>	Autoag, CV, CRMOX	O:3/4
YE27	Animal	2008	AMP, CEF	-	CRMOX	O:3/4
YE28	Animal	2008	AMP, CEF, SFT	-	CRMOX	O:3/4
YE29	Food	2008	AMP, CEF, SFT, SXT, TMP	-	CRMOX	O:3/4
YE30	Food	2008	AMP, CEF, SFT, SXT, TMP	-	-	O:3/4
YE31	Human	2008	AMP, CEF, SFT	-	-	O:3/4
YE32	Human	2005	AMP, CEF, SFT	-	-	O:3/4
YE33	Animal	2007	AMP, CEF, FOX, SFT	<i>inv</i>	PYZ	5a/1A
YE34	Environment	2007	AMP, CEF, FOX, SFT	<i>inv</i>	PYZ	5a/1A
YE35	Environment	2007	AMP, CEF	<i>inv</i>	PYZ	5b/1A
YE36	Animal	2007	AMP, CEF, SFT	-	PYZ	NT/1A
YE37	Environment	2007	AMP, CEF, SFT	-	PYZ	NT/1A
YE38	Environment	2007	AMP, CEF, FOX, SFT	<i>inv</i>	PYZ	NT/1A
YE39	Environment	2007	AMP, CEF	-	PYZ	NT/1A

(continue on next page)

Table 2. (continued) Results of antimicrobial resistance (AR), PCR of virulence genes and phenotypic tests of 60 *Y. enterocolitica* strains analyzed

Strain	Source	Date	AR	Virulence genes	Phenotypic tests	Serotype / Biotype
YE40	Environment	2008	AMP, CEF,SFT	<i>inv, ail, ystA</i>	-	O:3/4
YE41	Environment	2007	AMP, CEF,SFT	-	PYZ	NT/1A
YE42	Animal	2007	AMP, CEF,FOX, SFT, TET	-	PYZ	NT/1A
YE43	Environment	2007	AMP, CEF	-	PYZ	NT/1A
YE44	Environment	2007	AMP, CEF, FOX, SFT	<i>inv</i>	PYZ	NT/1A
YE45	Environment	2007	AMP, CEF	<i>inv</i>	PYZ	O:7/1A
YE46	Human	2008	AMP, CEF,SFT	<i>inv, ail, ystA</i>	-	O:3/4
YE47	Human	2008	AMP, CEF,SFT	<i>inv, ail, ystA</i>	-	O:3/4
YE48	Human	2008	AMP, CEF, SFT, SXT, TMP	<i>inv, ail, ystA</i>	-	O:3/4
YE49	Human	2009	AMP, CEF,SFT	<i>inv, ail, ystA</i>	Autoag, CV	O:3/4
YE50	Human	2009	AMP, CEF,SFT	<i>inv, ail, ystA</i>	-	O:3/4
YE51	Human	2009	AMP, CEF,SFT, TET	<i>inv, ail, ystA</i>	CV	O:3/4
YE52	Human	2010	AMP, CEF,SFT	<i>inv, ail, ystA</i>	-	O:3/4
YE53	Human	2010	AMP, CEF,SFT	<i>inv, ail, ystA</i>	-	O:3/4
YE54	Food	2011	AMP, CEF, FOX, SFT	<i>inv</i>	PYZ	5b/1A
YE55	Food	2011	AMP, CEF, FOX, SFT	<i>inv</i>	PYZ	5b/1A
YE56	Food	2011	AMP, CEF, FOX, SFT	<i>inv</i>	PYZ	5b/1A
YE57	Human	2011	AMP, CEF,SFT	<i>inv, ail, ystA, virF</i>	Autoag, CV, CRMOX	O:3/4
YE58	Human	2011	AMP, CEF,SFT	<i>inv, ail, ystA, virF</i>	Autoag, CV, CRMOX	O:3/4
YE59	Human	2011	AMP, CEF,SFT, TMP	<i>inv, ail, ystA</i>	-	O:3/4
YE60	Human	2011	AMP, CEF,SFT	<i>inv, ail, ystA</i>	-	O:3/4

AR: antimicrobial resistance; AMP: ampicillin; CEF: cephalotin; AMK: amikacin; FOX: cefoxitin; SFT: sulfamethoxazole; TET: tetracycline; SXT: sulfamethoxazole-trimethoprim; TMP: trimethoprim; Autoag: autoagglutination; CV: crystal violet; CRMOX: Congo red agar - magnesium oxalate; PYZ: pyrazinamidase activity at 37°C; NT: not serologically typed

Table 3. Resistance profiles of 60 isolated strains of *Y. enterocolitica*

Resistance profiles	Total	Serotype				
		O:3	5a	5b	O:7	NT/1A
AMP	1	1	-	-	-	-
AMP-CEF	12	8	-	1	1	2
AMP-SFT	1	1	-	-	-	-
AMP-CEF-SFT	26	23	-	-	-	3
AMP-CEF-SFT-TET	4	4	-	-	-	-
AMP-CEF-AMK-SFT	1	1	-	-	-	-
AMP-CEF-TET-SXT	1	1	-	-	-	-
AMP-CEF-SFT-TMP	1	1	-	-	-	-
AMP-CEF-SXT-TMP	1	1	-	-	-	-
AMP-CEF-SFT-TET-SXT	1	1	-	-	-	-
AMP-CEF-FOX-SFT	7	-	2	3	-	2
AMP-CEF-SFT-SXT-TMP	3	3	-	-	-	-
AMP-CEF-FOX-SFT-TET	1	-	-	-	-	1
Total	60	45	2	4	1	8

AMP: ampicillin; CEF: cephalotin; AMK: amikacin; FOX: cefoxitin; SFT: sulfamethoxazole; TET: tetracycline; SXT: sulfamethoxazole-trimethoprim; TMP: trimethoprim.

Cell lysis buffer (CLB – 50 mM Tris; 50 mM EDTA; pH 8.0 + sarcosyl 1%) and 25 µL of proteinase K (20 mg/mL stock (Invitrogen, Carlsbad, USA) were used for the cell lysis. The plugs were washed with sterile Ultrapure water (reagent grade type 1; Mili-Q, Darmstadt, Germany) and TE buffer (10 mM Tris; 1 mM EDTA; pH 8.0).

The plugs were digested with 10U/µL Not I (BioLabs, Ipswich, USA) and the electrophoresis system was CHEF-DR III (Bio-Rad, Hercules, USA). The gel was stained with ethidium bromide solution (0.5 µg/mL) and visualized by UV light. The molecular weight standard used was *Salmonella* serovar Braenderup H9812.

Data analysis was performed with BioNumerics version 4 (Applied Math, Austin, USA). Banding patterns were compared through the Dice coefficient by using the UPGMA method (unweighted pair group method with averages) to determine the similarity of bands and by adopting Tenover *et al.*'s [24] criterion in the definition of cluster types. A cut-off ≥ 85% was set forth in formation of groups considered genetically related. However, isolated strains with the 100% band patterns of similarity were considered to be representatives of the same pulsotype of PFGE [25]. For the assessment of the PFGE discriminatory capacity, the calculation of the Simpson's index of diversity was used [26].

Results

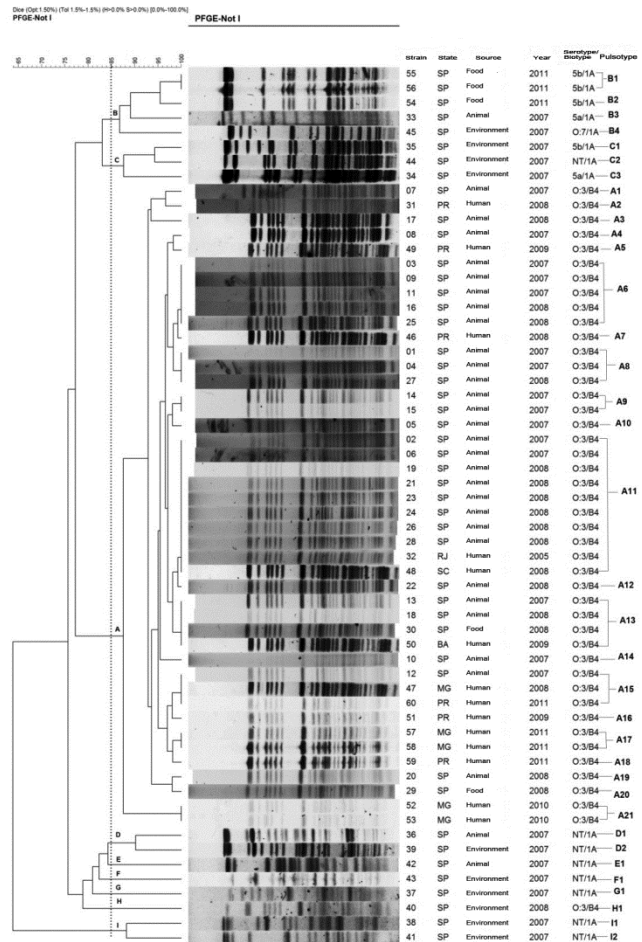
The data obtained in phenotypic tests, in antimicrobial resistance behavior (AR), and in PCR of virulence genes are listed in Table 2. Eleven positive strains in the phenotypic tests, except for pyrazinamidase activity, presented all virulence genes, including the plasmid gene *virF*, confirming the presence of the plasmid in nine animal and two human isolate strains, since the strain was only considered as the plasmid bearer when phenotypic tests and the PCR test for detection of *virF* gene were positive. The strains belonging to biotype 1A were positive for pyrazinamidase activity. Ten strains isolated from human sources (O:3/4) presented only three chromosomal virulence genes and nine strains belonging to biotype 1A presented the *inv* gene.

Antimicrobial susceptibility test revealed the high resistance of *Y. enterocolitica* to ampicillin (100%) and cephalotin (97%) in addition to the behavior of isolated strains in relation to other drugs. In terms of the resistance profiles of the strains, 8 (13%) were resistant to cefoxitin, 41 (68%) to sulfamethoxazole, 6 (10%) to sulfamethoxazole-trimethoprim, 5 (8%) to

trimethoprim, 7 (12%) to tetracycline, and 1 (2%) to amikacin. The predominant resistance profile was AMP-CEF-SXT (Table 3).

The PFGE analysis provided elements for the preparation of a dendrogram, consisting of nine clusters (A to I), allowing the grouping of the isolated strains with ≥ 85% similarity. Within the clusters, 36 isolated strains had pulsotype with 100% similarity, as shown in Figure 1. Cluster A comprised all serotype strains O:3 biotype 4 isolated from animal and human sources. The strains belonging to biotype 1A from food and environmental sources were spread throughout the others clusters (B to I).

Figure 1. PFGE dendrogram. Dendrogram built from the analysis of the bands, generating nine clusters (A to I) with similarity ≥ 85%, of 60 *Y. enterocolitica* strains obtained by PFGE.



SP: São Paulo; PR: Paraná; RJ: Rio de Janeiro; SC: Santa Catarina; BA: Bahia; MG: Minas Gerais.

Discussion

Phenotypic tests for detection of the plasmid were positive when *Y. enterocolitica* O:3 strains were incubated at 37°C. This is due to the plasmid characteristic related to this temperature [8]. In addition to the virulence plasmid, 11 pathogenic *Y. enterocolitica* O:3 strains had chromosomally encoded virulence genes (Table 2). The obtained results demonstrated the correlation of biotype and serotype (O:3 biotype 4) with pathogenic potential to susceptible hosts [27].

Ten human isolated strains showed only chromosomal virulence genes (Table 2). One of the hypotheses for this is the loss of plasmid, which can be easily lost at temperatures above 30°C, depending on the culture conditions [28]. However, it is not known how the plasmid may be lost below 30°C; further research is required [8]. Nine biotype 1A strains, both from food and environmental sources, and one from an animal source (Table 2), showed the *inv* gene. The strains of this biotype have generally been regarded as avirulent. The genes present in such strains match findings in Brazil [29] and worldwide [30], demonstrating the pathogenic potential of the representatives of this biotype.

Antimicrobial susceptibility test revealed the high resistance of *Y. enterocolitica* to ampicillin (100%) and cephalotin (97%) (Table 3), which has been commonly described in the literature [31]. This resistance is probably due to the presence of chromosomal genes *blaA* and *blaB* responsible for producing two β -lactamases, A and B [31]. Also, six (10%) strains were resistant to sulfamethoxazole-trimethoprim and seven (12%) to tetracycline, which are two antibiotics used in yersiniosis treatment [7,31]. One strain showed resistance to amikacin (AMP-CEF-AMK-SFT profile), an aminoglycoside, used in combination with other antibiotics for the treatment of extra-intestinal yersiniosis [2,7]. Such resistance has been described in strains isolated from animals in Brazil [32].

AMP-CEF-SFT was the resistance profile most commonly found in 26 strains of serotype O:3 and not serologically typed and no pattern could be established as to the serotype/biotype or origin. The strain YE42 (profile: AMP, CEF, FOX, SFT, TET) showed resistance to three different classes of antibiotics (cephalosporin, sulfonamide, and tetracycline), except for ampicillin and cephalotin (Table 3). Resistance to multiple antimicrobial agents was a rare event [33].

Low resistance to antibiotics was observed in isolated strains showing all virulence factors (Table 2).

Plasmid pYV plays no role in terms of participation in the antimicrobial resistance profile [34].

PFGGE analysis revealed nine clusters (A to I) grouping isolated strains with $\geq 85\%$ similarity (Figure 1). In these clusters, 36 pulsotypes were grouped, generating a discriminatory index of diversity of 0.957.

Cluster A comprised 21 pulsotypes (A1 to A21) with 87% similarity among isolated strains, all from bio-serotype 4/O:3 of animal and human sources. The high levels of similarity between the profiles match the results in the literature. They strongly support the hypothesis that pigs play an important role in the epidemiology of human sporadic *Y. enterocolitica* 4/O:3 infections [35].

The swine isolated strains belong to the same region and the same period of isolation, presenting a great similarity between them, featuring the epidemiological situation as a restrict niche. It is worth mentioning that A11 comprised two strains isolated from humans, one from Santa Catarina and another from Rio de Janeiro in 2008 and 2005, respectively. Based on this result, this pulsotype might circulate in Brazil, even in geographically distinct regions that are swine farming complexes. Additionally, pulsotypes A13 and A15 grouped strains isolated from human and animal sources, supporting the hypothesis.

It should be noted that swine farming products (meat, sausages, etc.) are typically marketed to various locations, whether or not geographically distant, promoting the circulation of *Y. enterocolitica* bio-serotype 4/O:3, with high phenotypic and genotypic similarity in various regions.

Based on this, two hypotheses may be proposed in order to explain the similarity of about 87% within cluster A. The first one is that these strains may be genetically related (same genetic lineage), and this difference may be due to some genetic events (point mutations) such as deletion or insertion of DNA or loss or gain of restriction sites [24]. The second hypothesis relates to the great natural similarity found between strains isolated from humans and from swine. Studies conducted in Finland and Germany showed that strains isolated from humans were almost indistinguishable from strains isolated from swine in those regions [35].

The biotype 1A strains isolated from food and the environment had greater diversity between fingerprints of their bands and were spread to the other clusters (B to I). This diversity may be related to different serotypes from this biotype; some of those strains could not be serologically typed [32,36].

Upon PFGE analysis of the strains with all virulence factors, it was observed that some of the samples were grouped in the same pulsotypes, such as A9, comprising YE14 and 15; A11, comprising strains YE 21, 23, 24, and 26; and A17, comprising the samples YE57 and 58. Based on these results, PFGE may be able to group strains bearing virulence genes. However, additional studies should be performed and further developed, provided that these samples are grouped within the same pulsotypes, rather than grouped according to their origins or their isolation source or serotype/biotype.

No direct correlation could be established between the pulsotypes of *Y. enterocolitica* and antimicrobial resistance patterns, as was observed in previous studies abroad [34].

Conclusions

Based on the results, *Y. enterocolitica* strains with bio-serotype 4/O:3, isolated from human and animal sources, showing the same genotypic profiles and bearing all virulence factors, circulate among several Brazilian states. These strains could become a potential threat to public health in Brazil as they have in Europe.

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References

- Mollaret HH, Bercovier H, Alonso JM (1979) Summary of the data received at the WHO reference Center for *Yersinia enterocolitica*. *Contr Microbiol Immunol* 5: 174-184.
- Bottone EJ (1997) *Yersinia enterocolitica*: the charisma continues. *Clinical Microbiol Rev* 10: 257-276.
- Fredriksson-Ahomaa M, Cernela N, Hächler H, Stephan R (2011) *Yersinia enterocolitica* strains associated with human infections in Switzerland 2001 – 2010. *Eur J Clin Microbiol Infect Dis* 31: 1543-1550.
- Ortiz Martínez P, Mylona S, Drake I, Fredriksson-Ahomaa M, Korkeala H, Corry JE (2010) Wide variety of bioserotypes of enteropathogenic *Yersinia* in tonsils of English pigs at slaughter. *Int J Food Microbiol* 139: 64-69.
- Falcão JP, Falcão DP (2006) Importância de *Yersinia enterocolitica* em microbiologia médica. *J Basic Appl Pharma Sciences* 27: 9-19.
- Fredriksson-Ahomaa M, Stolle A, Korkeala H (2006) Molecular epidemiology of *Yersinia enterocolitica* infections. *FEMS Immunol Med Microbiol* 47: 315-329.
- Fàbrega A, Vila J (2012) *Yersinia enterocolitica*: Pathogenesis, virulence and antimicrobial resistance. *Enferm Infecc Microbiol Clín* 30: 24-32.
- Zheng H, Sun Y, Mao Z, Jiang B (2008) Investigation of virulence genes in clinical isolates of *Yersinia enterocolitica*. *FEMS Immunol Med Microbiol* 53: 368-374.
- Wren BW, Tabaqchali S (1990) Detection of pathogenic *Yersinia enterocolitica* by the polymerase chain reaction. *Lancet* 3: 693.
- Magalhães VD, Ferreira JC, Barelli C, Darini ALC (2005) Eletroforese em campo pulsante em bacteriologia - uma revisão técnica. *Rev Inst Adolfo Lutz* 64: 155-161.
- Wauters G (1981) Antigens of *Yersinia enterocolitica*. In Bottone EJ, editor. *Yersinia enterocolitica*. Boca Raton: CRC Press. 41-53.
- Mollaret HH, Bercovier H, Alonso JM (1990) *Yersinia*. In: Le Minor L, Vérom M, editors. *Bactériologie Médicale*. Paris: Flammarion Médecine-Sciences. 298-315.
- Winblad S (1968) Studies on O antigen factors of "*Yersinia enterocolitica*". *Internat Symp on Pseudotuberculosis*, Paris, 1967. *Symp Series Immunobiol Standard* 9: 361-368.
- Asensi MD, Hofer E (1985) Pesquisa de *Yersinia enterocolitica* em pacientes reumáticos. I - Bacteriologia. São Paulo. *Rev Microbiol* 16: 260-268.
- Skurnik M, Bölin I, Heikkinen H, Piha S, Wolf-Watz H (1984) Virulence plasmid-associated autoagglutination in *Yersinia* spp. *J Bacteriol* 158: 1033-1036.
- Bhaduri S, Conway LK, Lachica RV (1987) Assay of crystal violet binding for rapid identification of virulent plasmid-bearing clones of *Yersinia enterocolitica*. *J Clin Microbiol* 25: 1039-1042.
- Riley G, Toma S (1989) Detection of pathogenic *Yersinia enterocolitica* by using congo red-magnesium oxalate agar medium. *J Clin Microbiol* 27: 213-214.
- Kandolo K, Wauters G (1985) Pyrazinamidase activity in *Yersinia enterocolitica* and related organisms. *J Clin Microbiol* 21: 980-982.
- Clinical and Laboratory Standards Institute (2011) Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational Supplement. Approved standard, CLSI document M100-S2131. CLSI: Wayne, PA, USA.

20. Rasmussen HN, Rasmussen OF, Andersen JK, Olsen JE (1994) Specific detection of pathogenic *Yersinia enterocolitica* by two-step PCR using hot-start and DMSO. *Mol Cell Prob* 8: 99-108.
21. Nakajima H, Inoue M, Mori T, Itoh KI, Arakawa E, Watanabe H (1992) Detection and identification of *Yersinia pseudotuberculosis* and *Yersinia enterocolitica* by an improved polymerase chain reaction method. *J Clin Microbiol* 30: 2484-2486.
22. Ibrahim A, Liesack W, Griffiths MW, Robins-Browne RM (1997) Development of a highly specific assay for rapid identification of pathogenic strains of *Yersinia enterocolitica* based on PCR amplification of the *Yersinia* heat-stable enterotoxin gene (*yst*). *J Clin Microbiol* 35: 1636-1638.
23. Centers for Disease Control (2006) One-Day (24-28 h) Standardized Laboratory Protocol for Molecular Subtyping of *Yersinia pestis* by Pulsed Field Gel Electrophoresis (PFGE). PulseNet USA. Atlanta: CDC. Available: http://www.cdc.gov/pulsenet/protocols/yersinia_Apr2006.pdf. Accessed 22 August 2011.
24. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, Swaminathan B (1995) Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 33: 2233-2239.
25. Johnson JK, Arduino SM, Stine OC, Johnson JA, Harris AD (2007) Multilocus Sequence Typing Compared to Pulsed-Field Gel Electrophoresis for Molecular Typing of *Pseudomonas aeruginosa*. *J Clin Microbiol* 45: 3707-3712.
26. Hunter PR, Gaston MA (1988) Numerical Index of the Discriminatory Ability of Typing Systems: an Application of Simpson's Index of Diversity. *J Clin Microbiol* 26: 2465-2466.
27. Paixão R, de Gobbi DD, Raimundo DC, Hofer E, Matte MH, Moreno AM (2012) Characterization of *Yersinia enterocolitica* 4/O:3 isolated from slaughterhouses and pork in the state of São Paulo, Brazil, and clinical cases in humans. *Adv Exp Med Biol* 954: 117-122.
28. Thoerner P, Bin Kingombe CI, Bögli-Stuber K, Bissig-Choisat B, Wassenaar TM, Frey J, Jemmi T (2003) PCR detection of virulence genes in *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* and investigation of virulence gene distribution. *Appl Environ Microbiol* 69: 1810-1816.
29. Paixão R, Moreno LZ, Gobbi DDS, Raimundo DC, Hofer E, Matté MH, Ferreira TSP, Gomes VTM, Costa BLP, Moreno AM (2013) Characterization of *Yersinia enterocolitica* Biotype 1A Strains Isolated from Swine Slaughterhouses and Markets. *The Scientific World Journal*. Article ID 769097.
30. Kraushaar B, Dieckmann R, Wittwer M, Knabner D, Konietzny A, Mäde D, Strauch E (2011) Characterization of a *Yersinia enterocolitica* biotype 1A strain harbouring an ail gene. *J Appl Microbiol* 111: 997-1005.
31. Bonke R, Wacheck S, Stuber E, Meyer C, Märklbauer E, Fredriksson-Ahomaa M (2011) Antimicrobial Susceptibility and Distribution of b-Lactamase A (*blaA*) and b-Lactamase B (*blaB*) Genes in Enteropathogenic *Yersinia* Species. *Microbial Drug Resist* 17: 575-581.
32. Falcão JP, Falcão DP, Silva AP, Malaspina AC, Brocchi M (2006) Molecular typing and virulence markers of *Yersinia enterocolitica* strains from human, animal and food origins isolated between 1968 and 2000 in Brazil. *J Med Microbiol* 55: 1539-1548.
33. Baumgartner A, Küffer M, Suter D, Jemmi T, Rohner P (2007) Antimicrobial resistance of *Yersinia enterocolitica* strains from human patients, pigs and retail pork in Switzerland. *Int J Food Microbiol* 115: 110-114.
34. Fredriksson-Ahomaa M, Meyer C, Bonke R, Stuber E, Wacheck S (2010) Characterization of *Yersinia enterocolitica* 4/O:3 isolates from tonsils of Bavarian slaughter pigs. *Lett Appl Microbiol* 50: 412-418.
35. Fredriksson-Ahomaa M, Stolle A, Siitonen A, Korkeala H (2006) Sporadic human *Yersinia enterocolitica* infections caused by bioserotype 4/O:3 originate mainly from pigs. *J Med Microbiol* 55: 747-749.
36. Souza RA, Pitondo-Silva A, Falcão DP, Falcão JP (2010) Evaluation of four molecular typing methodologies as tools for determining taxonomy relations and identifying species among *Yersinia* isolates. *J Microbiol Meth* 82: 141-150.

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